Mutants of Yeast Sensitive to Ultraviolet Light

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Six uvr mutants of Saccharomyces cerevisiae with hypersensitivity to ultraviolet (UV) light were isolated after mutagen treatment with ethylmethanesulfonate. UV sensitivity ranges from moderate to extreme, and four of the mutants are also sensitive to nitrous acid. Ranking in terms of UV sensitivity does not parallel ranking in terms of nitrous acid sensitivity. Homozygous diploid mutant strains are somewhat less sensitive than the corresponding haploids. All mutations are recessive. None of the mutants is sensitive to γ rays, and each shows photoreactivation after UV radiation. Complementation tests and tetrad analysis indicate that each strain represents mutation in a different gene. Two of the uvr genes are linked, and two others are centromere-linked.

The paper of Clark and Margulies (2) reporting the isolation of Escherichia coli mutants defective in genetic recombination, and their finding that such mutants were also sensitive to ultraviolet (UV) light, put the study of recombination in an entirely new perspective. Their discovery linked the process of recombination with enzymatic repair of irradiated deoxyribonucleic acid (DNA) and suggested new approaches for joint biochemical and genetic analysis of one of the basic characteristics of living organisms. Several other investigators have since isolated radiation-sensitive mutants of E. coli which are defective in recombination (9, 13). Radiation-sensitive mutants have also been found in four species of fungi: Ustilago (8), Aspergillus (W.B. Lanier and R. W. Tuveson, Genetics 54:345, 1966), Saccharomyces (11), and Schizosaccharomyces (4). Since these fungi possess a meiotic cycle essentially like that of most higher organisms, it is clearly of interest to inquire into the relation between radiation sensitivity and recombination in these eucaryotes. As a preliminary step in such a study, mutants of bakers' yeast (Saccharomyces cerevisiae) were isolated which showed hypersensitivity to UV light. Some characteristics of these uvr mutants are described in this paper.

MATERIALS AND METHODS

Yeast strains. S288C: α, standard prototrophic strain obtained from R. K. Mortimer; S2-2c: a hist, leu; S1-7b: a, prototrophic strain; S4-4a: a hist, adh. The latter three strains were obtained by ascus dissections from various crosses. All these strains are haploid.

Growth media and conditions. YEP medium contained 1% yeast extract, 2% Bacto Peptone, and 2% glucose. Minimal medium was Difco Yeast Nitrogen Base without amino acids. For plates, 2% agar was added. Sporulation medium contained 0.98% potassium acetate, 0.25% yeast extract, 0.1% glucose, and 1.5% agar.

Cells from refrigerated agar slants of YEP were usually inoculated into 4 ml of YEP in tubes (16 by 125 mm) which were held in an inclined test tube rack in a New Brunswick G25250 recirculating shaker. Incubation was at 30°C for 3 days with vigorous shaking. Plate counts were made after 4 or 5 days of growth.

Experimental procedures. To produce uvr mutants, S288C was grown in 10 ml of YEP in a 50-ml flask with shaking for 3 days at 30°C. The culture was washed twice with water, resuspended in 10 ml of phosphate buffer (pH 6.5) to which 0.3 ml of ethylmethanesulfonate (EMS) was added, and incubated with shaking for 1 hr. It was then washed once and resuspended in 15% sodium thiosulfate for 15 min, after which it was washed twice more. Viability after comparable EMS treatment in other experiments was about 10%. A 10-fold dilution into YEP was made, and 1 ml of this dilution was added to each of 20 tubes (13 by 100 mm) which were incubated overnight in a roller drum. Appropriate dilutions of each tube were spread on YEP plates to yield about 200 colonies per plate, and replicas were made onto minimal and YEP plates after 3 days of growth. These replicas were exposed to about 2,000 ergs/mm² of UV before being placed in the incubator for overnight growth. Presumptive sensitive clones, which appeared to suffer excessive killing on the replica plates, were picked from the master plates for further testing. Six uvr mutants were so obtained, each from a different tube, from about 10,000 colonies examined. None of the uvr strains is a petite mutant.

Survival after UV irradiation was determined by plating appropriate dilutions of 3-day YEP cultures onto YEP plates before exposure of the plates to UV. For the irradiation, a General Electric G8T5 lamp in a Gates Raymaster reflector was used, with the...
acetate buffer solution 4.5-ml washed source. Cobalt-60 were withdrawn at 5-min intervals and added to 4.5 ml of 0.05 M tris(hydroxymethyl)aminomethane buffer (pH 8) to stop the reaction. Dilutions were plated on YEP. For viability plating without treatment, a second 4.5-ml sample was handled in exactly the same way, except that 0.5 ml of water was added instead of the NaNO₂ solution.

For exposure to γ rays, 1 ml of appropriate dilutions in phosphate buffer of washed 3-day YEP cultures was put into Pyrex tubes (13 by 100 mm) held in a wire rack. The samples were exposed to γ rays from a cobalt-60 source. The dose rate was 655 rad per min, as measured by ferrous sulfate dosimetry (16).

Scoring for UV sensitivity or resistance of spore clones obtained by ascus dissection was done by suspending in 0.5 ml of water about 2 × 10⁶ cells from freshly grown (1- or 2-day) cultures on YEP plates. A 3-mm glass rod was dipped into the suspension and then touched to the surface of a YEP plate, leaving a uniform layer of cells. The spots were placed in a regular grid pattern which accommodated the four spore clones from each of 16 asci on a single plate. Spots of the two parental uvr strains and of S288C were also put on the plate as controls. Plates were then exposed to 1,000 ergs/mm² of UV and incubated for 2 days in the dark. Sensitive clones ordinarily showed complete killing of all cells of the spot, or a few cells survived to form colonies (usually less than 10), whereas resistant clones showed confluent or almost confluent growth. This method of scoring allows a clear-cut distinction to be made between sensitive and resistant segregants, and was used in preference to replica plating, where scoring was often equivocal because the large amount of overlayering of cells prevented their exposure to the full dose of UV.

Ascus dissections were performed by the method of Johnston and Mortimer (10).

RESULTS

Survival curves after UV irradiation for six uvr strains isolated as described in the previous section are shown in Fig. 1. The curves for uvr-4 and uvr-9, the most sensitive strains, are essentially identical, whereas the other four strains exhibit different, and less extreme, degrees of sensitivity. The tails on the uvr-4 and uvr-9 curves may represent a UV-resistant fraction produced by reversion. Broad shoulders are characteristic of the uvr-10 and uvr-11 curves, which resemble the parental strain S288C in this respect.

Survival of haploids was compared with homozygous diploids. Each of the uvr strains (α) was crossed with strain S2–2c (α), and zygotes were isolated by micromanipulation. After sporulation of the resulting diploid strains, prototrophic uvr isolates of mating type a were obtained by ascus dissection. Regular 2:2 segregation of the resistant and sensitive characters was obtained for each uvr strain, except for an occasional aberrant segregation of 3:1 or 4:0. The a uvr isolates were mated with the original strains to obtain diploid strains homozygous for each uvr allele. Survival curves after UV irradiation for the diploid strains are shown in Fig. 2. Except for uvr-4, the homozygous diploids are somewhat less sensitive to UV than the haploids. The curves of the four most sensitive strains show a slight shoulder at about 30 ergs/mm².

Survival after nitrous acid treatment is shown in Fig. 3. The extreme nitrous acid sensitivity of uvr-10, the strain least sensitive to UV, is most striking. Strains uvr-8 and uvr-11 have the same resistance as the parental strain, whereas the extremely UV-sensitive uvr-4 and uvr-9 have intermediate nitrous acid sensitivity.

None of the uvr strains is especially sensitive to γ rays (Fig. 4). The higher survival of uvr-4 after γ irradiation seems characteristic of that strain, and may be related to its anomalous genetic behavior in certain crosses, which sug-
homozygous uvr enzyme, as resistant as type diploid. Each mating or diploid that suggests that it may consist of haploid plus aneuploid or diploid cells.

Heterozygous diploid strains, obtained by mating each uvr strain with strain S1-7b, were as resistant to UV as a uvr+ homozygous wild-type diploid. Each uvr allele is therefore recessive. All uvr strains also possess a functional photoreactivating enzyme, as survival after UV irradiation was enhanced by exposure to light from “cool-white” fluorescent tubes.

Complementation tests were made in an effort to determine how many loci are represented in the collection of the six mutant strains. Diploids from all pairwise crosses of the mutants were made, using haploids of the proper mating type obtained from crosses with S2-2c and S4-4a. Survival of the diploids, determined after a constant dose of 500 ergs/mm², was in the range of 50 to 100%. The experiment was repeated with a dose of 1,000 ergs/mm², which resulted in survivals in the range of 7 to 53%. In both experiments, the wild-type diploid controls showed somewhat higher survival than most of the mutant combinations, but this difference was not extreme, and could be due to genetic differences other than the uvr alleles or to uncontrolled experimental variables. All combinations thus appear to complement, since none resulted in diploids with sensitivities approaching those of the homozygous uvr diploids. This fact, coupled with the differences which were observed in UV and nitrous acid sensitivity, indicates that each strain represents mutation in a different gene. This contention is borne out by the results of ascus analysis of pairwise crosses between the uvr strains (Table 1). Crosses involving two alleles at the same locus should have produced only parental ditype ascii which contained four sensitive spores. In most cases, parental ditype and nonparental ditype ascii were formed in approximately equal proportions, thus giving no indication of linkage. The genes uvr-4 and uvr-3 are linked, however, about 15 map units apart.

Analysis of tetrads from the crosses of each uvr strain to strain S2-2c is presented in Table 2. The allele le1 is closely linked to the centromere of chromosome VII. This makes it possible to determine whether any uvr gene is centromere-linked, since in such a case the frequency of tetratypes would be less than two-thirds (6). The analysis shows that uvr-9 and uvr-10 are linked to a centromere. None of the genes shows linkage to le1, as the P:N ratio is in no case significantly different from 1:1. However, uvr-5 might be linked to his3 on chromosome V, since there

**Fig. 2. Survival after UV irradiation of diploid homozygous uvr strains.**

**Fig. 3. Survival after nitrous acid treatment of the uvr strains.**
was a barely significant excess of P tetrads. In the case of \textit{uvr-11}, there is a significant excess of tetratypes over two-thirds, which could be taken as an indication of chiasma interference (6).

**DISCUSSION**

The \textit{uvr} yeast mutants described in this paper seem to be analogous to the bacterial mutants defective in "dark repair" (for review, see reference 14). An important first step in dark repair is the excision from DNA of thymine dimers formed by UV (1, 15). Later steps probably involve repair synthesis, with the single strand of DNA opposite the gap serving as a template (12). It may be difficult to study the biochemical events involved in dark repair of yeast DNA since thymine-requiring mutants, which would allow specific labeling of the DNA, have not so far been obtained. However, some information on the order of events in the repair process might be obtained by comparing the survival curves of double mutants with those of the parental single mutants. On the assumption that the \textit{uvr} mutations are nonleaky and affect the same pathway, the survival curve of a double mutant should be similar to that of the single mutant which represents the earlier step in the repair process. If the mutants are leaky, or if they affect different pathways in repair, the double mutant would be expected to show more extreme sensitivity than either component single mutant.

It has been proposed that the dark repair process may be a general error-correcting mechanism which acts on local distortions of the DNA.

**Table 1. Tetrad analysis of pairwise crosses of \textit{uvr} strains**

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<th>\textit{uvr} strain</th>
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<th>8</th>
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<tr>
<td>\textit{uvr} strain</td>
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<td>7:3</td>
<td>4:2</td>
<td>9:4</td>
<td>2:4</td>
<td>3:3</td>
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<td></td>
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<td>19</td>
<td>19</td>
<td>14</td>
<td>21</td>
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\textit{uvr} strains were scored as parental ditypes; those producing two sensitive and two resistant, as non-parental ditypes; and those producing three sensitive and one resistant, as tetratypes. Only complete ascii were scored. The number of parental ditype (P), nonparental ditype (N), and tetratype (T) ascii is given as $P: N: T$.

**Table 2. Tetrad analysis of crosses of \textit{uvr} strains with strain S2-2c (a le1 his1)**

<table>
<thead>
<tr>
<th>\textit{uvr} strain</th>
<th>\textit{uvr-le1}</th>
<th>\textit{uvr-his1}</th>
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<tbody>
<tr>
<td>\textit{uvr} strain</td>
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\textit{P}, parental ditype; \textit{N}, nonparental ditype; \textit{T}, tetratype tetrads.

6 Tetratypes significantly less than two-thirds ($x^2 = 10.11$, $P < 0.001$; and $x^2 = 4.88$, $P < 0.05$ for \textit{uvr-9} and \textit{uvr-10}, respectively).

7 Tetratypes significantly greater than two-thirds ($x^2 = 7.63$, $P < 0.01$).

\textit{P}:\textit{N} ratio just significantly different from 1:1 ($x^2 = 3.85$, $P = 0.05$).
helix (7, 15). This idea is supported by the finding that E. coli B_{r1}, which is extremely sensitive to UV, is also sensitive to nitrogen mustard and X rays (7), agents whose primary effect on DNA does not involve production of thymine dimers. Nitrous acid, like nitrogen mustard, has been reported to cause cross-linking of complementary DNA strands (3). Since the ranking of the uvr yeast mutants with respect to UV and nitrous acid sensitivity is so different, it is likely that different enzymatic reactions are involved in the efficient repair of UV and nitrous acid lesions, though the repair enzymes may all be components of one general dark repair system.

Mutants sensitive to γ rays (which should also be sensitive to X rays) were not found among the six described above. X-ray sensitive mutants of yeast are known, however (11; Resnick, personal communication). It will be especially interesting to test such mutants for their effect on recombination, since X-ray sensitivity appears to be characteristic of the E. coli mutants defective in this process (9, 13).

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LITERATURE CITED